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Both cladribine and alemtuzumab may effect multiple sclerosis via B cell depletion

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ABSTRACT

OBJECTIVE. To understand the efficacy of cladribine treatment in multiple sclerosis (MS) through analysis of lymphocyte subsets collected, but not reported, in the pivotal phase III trials of cladribine and alemtuzumab induction-therapies.

METHODS. The regulatory submissions of the CLARITY (NCT00213135) cladribine and CARE-MS I (NCT00530348) alemtuzumab trials were obtained from the European Medicine Agency through Freedom of Information requests. Data was extracted and statistically analysed.

RESULTS. Either dose of cladribine (3.5mg/kg; 5.25mg/kg) tested in CLARITY reduced the annualised relapse rate to 0.16-0.18 over 96 weeks, and both doses were similarly effective in reducing the risk of MRI lesions and disability. Surprisingly, however, T cell depletion was rather modest. Cladribine 3.5mg/kg depleted CD4⁺ cells by 40-45% and CD8⁺ cells by 15-30%, whereas alemtuzumab suppressed CD4⁺ cells by 70-95% and CD8⁺ cells by 47-55%. However, either dose of cladribine induced 70-90% CD19⁺ B cell depletion, similar to alemtuzumab (90%). CD19⁺ cells slowly repopulated to 15-25% of baseline before cladribine re-dosing. However, alemtuzumab induced hyper-repopulation of CD19⁺ B cells 6-12months after infusion, which probably forms the substrate for B cell autoimmunities associated with alemtuzumab.

CONCLUSIONS. Cladribine induced only modest depletion of T cells, which may not be consistent with a marked influence on MS, based on previous CD4⁺ T cell depletion studies. The therapeutic drug-response relationship with Cladribine is more consistent with lasting B cell depletion and, coupled with the success seen with monoclonal CD20⁺ depletion, suggests B cell suppression could be the major direct mechanism of action.

GLOSSARY

Alemtuzumab (ALEM)

Analysis of variances ANOVA

Cladribine (CLAD)

Disease modifying treatments (DMT)

Freedom of Information (FOI)

Multiple sclerosis (MS)

Natural killer (NK)

People with relapsing MS (pwRMS)

Secondary autoimmunity (SAI)

Standard Deviation (SD)

Standard Error of the Mean (SEM)

INTRODUCTION

Multiple sclerosis (MS) is a central nervous system demyelinating disease responding to immunosuppression¹. Pulsed induction-therapies with cladribine (CLAD)^{2,3} and alemtuzumab (ALEM)⁴⁻⁶ can induce long-term remission^{3,6}, whilst reducing risks of a permanent immunosuppressive-state through continuous drug-use⁷. Pivotal trials of an oral CLAD, 2-chlorodeoxyadenosine triphosphate, prodrug⁸ and ALEM, CD52-depleting antibody⁹, suggest that both drugs have comparable clinical efficacy in controlling relapses²⁻⁵, but markedly-different side-effect profiles^{2,4,5}. Whilst both drugs induce lymphocyte depletion⁸⁻¹¹, only ALEM causes significant secondary B cell autoimmunity (SAI) in people with MS^{6,9}. It was suggested that CLAD may create a cancer risk², which in the absence of additional trial data caused regulators to refuse licencing and halted CLAD development in 2011¹². However, a subsequent CLAD trial¹³ and meta-analysis¹⁴ indicated that the CLAD-associated cancer frequency was no different to natural aging or other pivotal MS-drug trials¹⁴. This suggested that even in the absence of oral CLAD, injectable generic CLAD, may still have value in treating active MS^{15,16}.

Whilst the mechanism of action of CLAD in MS is unclear¹⁷, efficacy of ALEM has been attributed to CD4⁺ T cell deletion and relative-sparing of T regulatory cells^{9,18} and SAI to homeostatic T cell proliferation and lack of thymic repopulation¹⁹. Whilst immune-reconstitution kinetics after ALEM have been reported^{10,18,20}, the lymphocyte-subset of pivotal CLAD/ALEM trials were only partially disclosed^{2,4,5}, yet meeting-abstracts indicated that lymphocyte subset data was collected and analysed years ago^{21,22}. We hypothesised that differences in the CLAD/ALEM lymphocyte repopulation kinetics may offer insights into the efficacy of CLAD and adverse-effect profile of ALEM.

METHODS

Standard Protocol Approvals, Registrations, and Patient Consents.

Freedom of Information (FOI) requests to the European Medicines Agency (EMA) for the full regulatory submissions of phase III “CLAD tablets treating multiple sclerosis orally” (CLARITY; NCT00213135)² and “Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis, study one” (CARE-MS I; NCT00530348)⁴ trials were made. Whilst these trials were recruited following ethical approval of the trials and informed consent, as previously reported^{2,4}, no specific ethical approval was obtained or required to view and use these “public domain” documents. The details of participants were anonymous. Information relevant to: study design, setting, participants, eligibility, variables, randomization, blinding, study size, bias reduction, flow diagrams of participants and the CONSORT and STROBE reporting guidelines can be obtained from the original CLARITY² and CARE-MS I⁴ publications.

Trial Designs

The full details of the trials have been reported previously^{2,4}. Briefly, in the 96 week CLARITY trial people with relapsing MS (pwRMS) were randomized 1:1:1 to receive either placebo, or one of two doses of oral CLAD. Patients were given tablets containing either 10mg/day (60-69.9kg body weight) or 20mg/day (70-79kg body weight) CLAD prodrug administered for 4-5 days in weeks 0 and 5 (year 1) and weeks 48 and 52 (year 2) to result in a total cumulative dose of 3.5mg/kg. Those randomized to the 5.25mg/kg arm were given additional doses in weeks 9 and 13². In the CARE-MS I, pwRMS were randomly allocated 1:1 to receive either interferon β -1a (Rebif 44mg tiw) or ALEM 12mg/day on days 1-5 in year 1, followed by 12mg/day on days 1-3 one year later⁴.

Freedom of Information Requests

After termination of the commercial development of oral CLAD in 2011, and subsequent conversations with the UK Medicines and Healthcare products Regulatory Agency about approaches to develop generic CLAD, the full regulatory submission of the CLARITY trial² was obtained through a FOI request

(Submitted May 2013, obtained November 2013). The dataset was provided in portable document format (pdf). Files containing relevant data were identified and converted into Microsoft Excel spreadsheets using a pdf parser developed on a Python 2.7 platform at the MidPlus computational facilities at Queen Mary University of London (code available on request). The converted data was validated by comparing sample records between pdf and spreadsheet versions of the files. In addition we obtained redacted copies of the regulatory submission of the CARE-MS I trial⁴. The data was provided in pdf batches during the third and fourth quarter 2016. However primary (raw) white cell counts were not included in this package. The data presented here were therefore extracted from the tabulated documents.

Lymphocyte Phenotyping data

In both CLARITY and CARE-MS I, lymphocyte subsets were analysed using flow cytometry. Both datasets included the following: CD3⁺, CD4⁺ and CD8⁺ T cells, CD19⁺ B cells, CD16⁺/CD56⁺ (Natural Killer NK) cells; CD4⁺/CD45RA⁺ (naïve T helper) cells; CD4⁺/CD45RO⁺ (memory T) cells. The data was presented as absolute numbers/unit volume in both studies.

Statistical Analysis

Statistical analysis comparing two or more unpaired independent nominal variables was performed using chi-square test for heterogeneity. If statistical significance was detected for comparison of more than two variables post-hoc chi-square test for heterogeneity with Bonferroni correction for multiple comparisons was applied. Data is represented as mean \pm standard deviation (SD) unless described otherwise. For comparison of unpaired independent continuous variables two-tailed Student's t test for unpaired samples assuming unequal variances was used. For comparison of more than two unpaired independent continuous variables one way analysis of variances (ANOVA) with post-hoc Tukey was used. It was evident that the whole population was not analysed at every time point as indicated in the text, no adjustments for such missing data were made.

RESULTS

In total, 309/1326 pWRMS from the CLARITY trial² had lymphocyte subsets analysed (Figure 1), with n=101 in the placebo arm, n=103 in the CLAD 3.5mg/kg arm, and n=105 in the CLAD 5.25mg/kg arm (Supplementary Table e-1 and Table e-2). There was no significant difference for any demographics or clinical characteristics among the study arms (Table e-2). Importantly, compared to placebo, both doses of CLAD caused significant and comparable ($p=0.953$, ANOVA post-hoc Tukey) reduction in the annualised relapse rate (relative reduction compared to placebo 55% and 61% for CLAD 3.5mg/kg and 5.25mg/kg, respectively, over 96 weeks) and MS-related magnetic resonance imaging parameters (Table e-2). The sample was representative of the overall population in the CLARITY study².

Full blood count

Following administration of CLAD only minor and non-significant depletion of platelets and red blood cells occurred. However, there was significant ($p<0.01$) depletion of the total leukocyte population within a month of the second cycle of both doses of CLAD (Figure 1A). As expected, lymphocytes (Figure 1B)² were markedly depleted over the 96 weeks observation period ($p<0.01$) compared to more subtle influences on monocytes (Figure 1C), polymorphonuclear neutrophils (Figure 1D)², eosinophils (Figure 1E) and basophils (Figure 1F). Following lymphocyte depletion with two doses of CLAD there was further depletion following use of two additional doses (Figure 1B).

Lymphocyte phenotyping

Whilst reduction of CD3⁺ T cells showed some dose-dependency (Figure 2A), depletion of CD19⁺ B cells was very similar with both dosing schedules (Figure 2B). B cells numbers dropped markedly following the first course of CLAD with a nadir (85-90% depletion) at the time of the second dose of cycle one. B cells did not drop further following administration of additional CLAD doses as part of the 5.25mg/kg schedule and recovered to 30% baseline prior to the second treatment cycle after 48 weeks, which

again led to significant depletion (80% baseline; Figure 2B). In contrast to the B cell population, a dose-response effect with CD4⁺ and CD8⁺ T cells was detected (Figure 2, C and D). In the 3.5mg/kg group CD4⁺ T cell depletion by 20% occurred following the first dose, and by 45% following the second dose of cycle one. This level of depletion was maintained until the second cycle of treatment was given, which led to a maximum depletion by 60% baseline, over the duration of the study (96 weeks). In the 5.25mg/kg group depletion was more pronounced, by 70% of baseline after the first cycle, remaining at this level until the end of the observation period (Figure 2C). CD8⁺ T cells followed a rather similar kinetic as CD4⁺ T cells though depletion was overall less pronounced. In the 3.5mg/kg group CD8⁺ T cells were reduced by 30% and 40% baseline following treatment cycles 1 and 2, respectively. In the 5.25mg/kg group CD8⁺ T cells were reduced by 50% baseline after the first treatment cycle with no further depletion following the second cycle. Again similar to CD4⁺ T cells, CD8⁺ T cells did not show any significant recovery during the observation period (Figure 2D). The two CD4⁺ T cell subsets analysed (naïve, i.e. CD45RA⁺ and memory, i.e. CD45RA⁻) T cells were both affected and revealed rather similar kinetics of depletion and recovery (Figures 2E, 2F). In the 3.5mg/kg group naïve T cells were depleted by 45% and 60% baseline following treatment cycles 1 and 2, respectively (Figure 2E) with reductions in memory T cells being slightly less pronounced (Figure 2F). Maximum reduction of naïve T cells in the 5.25mg/kg group was by nearly 80% baseline within 12 weeks after treatment cycle 1, subsequently remaining at 70% throughout the remainder of the study, whilst memory T cells described a similar curve of depletion and (minor) recovery at 60-65%. Three months after treatment initiation naïve CD8⁺ T cells in the 3.5mg/kg group were reduced by 26.5% and memory CD8⁺ T cells by less than 10% of baseline levels. No difference in CD3, CD4, CD8, or CD19 lymphocyte counts was detected between the patient cohorts remaining free of relapses and patients who developed at least one relapse (Figure 3).

Comparison of lymphocyte kinetics after cladribine and alemtuzumab administration

Although the demographics of pwRMS were different between CLARITY² and CARE-MS I⁴ studies (Table e-1), both CLAD and ALEM exhibited high efficacy²⁻⁵. However, analysis of lymphocyte subsets following CLAD and ALEM treatment revealed several differences (Figure 4). (i) One month after the first treatment cycle with ALEM CD4⁺ T cells were almost completely (by over 95% baseline) depleted (Figure 4A). Even one year after treatment, just before the second treatment cycle, CD4⁺ T cells remained depleted by over 70% of baseline. The 5.25mg/kg dose of CLAD led to reduction of CD4⁺ T cells by 70% baseline within three months, with very little recovery until the second treatment cycle nearly nine months later. (ii) With the 3.5mg/kg dose of CLAD, which was clinically as effective as the 5.25mg/kg dose^{3,4} (Table e-2), depletion of CD4⁺ T cells during year one was much less pronounced than with ALEM or CLAD 5.25mg/kg. The gap between the CD4⁺ T cell depletion curves induced by the two different CLAD dosing schedules only narrowed during year 2, though neither dose ever caused a nadir similar to ALEM (reduction by >90% baseline). (iii) The kinetics of CD8⁺ T cell depletion was comparable for both ALEM and CLAD (high and low doses) to their respective CD4⁺ T cell behaviour (Figure 4B). (iv) Whilst ALEM, as well as both doses of CLAD, induced depletion of NK cells, this cell type rapidly recovered following ALEM treatment, and were even slightly above baseline at 6 and 12 months. In contrast, both doses of CLAD caused pronounced and virtually identical NK cell depletion, by 47% of baseline, within nine weeks of treatment cycle 1 (n= 71-72) (Figure 4C). (v) The most striking difference became evident comparing the effect of ALEM and CLAD on the CD19⁺ B cell population (Figure 4D). Whilst the level of depletion was initially similar between ALEM and both doses of CLAD, the repopulation characteristics were very different. After CLAD, B cells slowly repopulated, remaining significantly below their baseline level until the second treatment cycle reduced this subset yet again. In contrast, B cell numbers after ALEM repopulated back to baseline within less than six months and then hyper-repopulated well above baseline by nine and 12 months (Figure 4D). Surprisingly, neither of the peer-reviewed phase III trial reports of ALEM in MS provided any indication of the latter, but only reported that B cells reach normal levels six months after drug administration^{4,5}.

DISCUSSION

We analysed the lymphocyte repopulation kinetics following depletion with oral CLAD prodrug and intravenous ALEM using datasets from their regulatory submissions. Using data mining of these publicly available documents it was possible to obtain additional information not previously published in peer-reviewed papers. This may provide value for understanding the mechanisms of action and side-effect profiles of these disease modifying therapies (DMT), which have focused on the pathogenic role of T cells²³. However, the therapeutic drug-response relationship with CLAD is perhaps more consistent with lasting B cell depletion of both doses and helps create a focus on the role of the B cells in control of MS, which may or may not act via T cells²⁴. The data obtained with alemtuzumab is consistent with previous smaller scale studies^{10,25}

There is unequivocal evidence from the marked induction of disease following haematopoietic stem cell treatment that relapsing MS is due to aberrant function of the immune system²⁶. CLAD induced a modest depletion in T and NK cells, but a more marked depletion of B cells. This was consistent with the known selective lymphocyte depleting effect, because they express high levels of deoxycytidine kinase that phosphorylates CLAD, a deoxyadenosine analogue, to cytotoxic 2-chlorodeoxyadenosine triphosphate⁸. Although causality was not proven, the phase III trial data may suggest that lymphocyte depletion contributes to the reduction in disease activity in pWRMS^{2,3}. CLAD also effectively inhibited the evolution from a clinically-isolated syndrome of demyelination to definite MS¹³. Most of the ongoing disease activity in that trial occurred within the first three months of the study¹³. Based on the data presented here, maximum depletion using the dosing schemes employed takes between one and three months. Re-baselining of efficacy data at three months after first treatment course would have made the suppression of disease activity even more impressive¹³. Furthermore, the slower depletion kinetics of CLAD probably contributes to the lack of administration-related reactions with CLAD that are reported in approximately 90% of pWRMS treated with ALEM^{4,5}.

Whilst MS has commonly been considered to be a CD4⁺ T cell (Th1/Th17) mediated disease²³, a concept supported by the marked (95%) CD4⁺ T cell depletion induced by ALEM, CD4⁺ T cell depletion *in isolation* has arguably failed to control MS^{24,27}. Depletion of CD4⁺ T cells using a CD4-specific antibody, dosed to maintain CD4⁺ T cell numbers above 250 cells/mm³ (equivalent to a depletion of about 70%) to limit immunodeficiency thresholds²⁷, did not effectively suppress the development of new lesions on MRI, and was considered to have failed in MS²⁷. Based on our analysis, the 45-50% depletion of CD4⁺ T cells achieved by CLAD 3.5mg/kg, leaves these cells above the threshold required for optimal disease inhibition in both CD4 T cell-mediated experimental autoimmune encephalomyelitis in animals²⁸, and MS²⁷. Therefore, CD4⁺ T cell depletion may not account for the efficacy of this drug, in terms of relapse reduction and its effect on new MRI lesions, given a strong treatment effect of CLAD was already detectable within the first year of treatment^{2,3,13}.

The perceived failure of CD4 depleting monoclonal antibody led some groups to speculate that CD8⁺ T cells may be the pathogenic drivers of MS as suggested by the predominance of this cell type in MS lesions²⁹. Again, given the depletion threshold established for CD4 depletion^{27,28}, we hypothesize this is unlikely to be a key mechanism of action of CLAD, given CLAD 3.5mg/kg had only a minor (15-28%) CD8⁺ T cell depleting effect in year 1, when a strong disease modifying effect was already evident^{3,13}. However, again this comes with the proviso an effective threshold of CD8⁺ T cell depletion required for effective immunotherapy in MS, has yet to be defined. In contrast, ALEM markedly depletes CD8⁺ T cells. This, coupled with depletion of other immune subsets, may potentially contribute to the high number of reported infections that occurred in 77% of pWRMS participating in the CARE-MS I trial, compared to only 48% in the CLARITY trial^{2,4}. In addition, ALEM is more likely to effect CD8⁺ regulatory/suppressor cell responses that can control tolerance and potential SAI induction²⁸.

Whilst it has been shown that the cancer rate in pWRMS on CLAD was no different compared to all contemporaneously-licensed DMT¹⁴, NK cells represent an important part of cancer immunosurveillance. As CLAD induced a modest depletion of NK cells, vigilance is therefore advised over the intermediate and longer-term to truly establish safety, whether or not oral CLAD becomes a licensed DMT for pWRMS, or a generic preparation of CLAD is being used off-label^{16,30}.

CLAD induced a marked and long-lasting CD19⁺ B cell-depletion that did not reach baseline levels within the 12 month treatment cycle, a repopulation-kinetic evidently contrasting with ALEM. Whilst the capacity of ALEM for hyper-repopulation of B cells has been reported previously^{10,20}, it was subsequently largely ignored, notably in the phase III trial reports^{4,5}. We have recently reported that CD8⁺ T cell-depletion by CD52-specific antibodies can block immunological tolerance induction²⁸, suggesting it could contribute to the rapid hyper-repopulation of B cells that may be the prelude of SAI. Such hyper-repopulation of CD19⁺ B cells is not a feature of treatment with CLAD or CD20-specific B cell-depleting therapies³¹ neither of which are associated with B cell-driven autoimmunities^{2,32} or significant T cell depletion³¹. Following ALEM, the CD19⁺ B cell hyper-repopulation into the blood is due to the accumulating immature B cells, probably from the bone marrow, where they differentiate into mature/naïve B cells²⁰. When this occurs in the absence of T cell regulation, previous studies have shown that B cell autoreactivity can develop, and this may be a cause of SAI following use of ALEM in pWRMS^{33,34}. Previously, it has been shown that the apparent increase in the number of CD19⁺ B cells generated by the over-production of immature/mature B cells following ALEM infusion, masks a long-lasting depletion of CD19⁺/CD27⁺ memory B cells²⁰, which also occurs to varying levels with other agents that control MS^{24, 31}, suggesting that these cells may be important in disease control by DMT²⁴. However, it must be recognised that there are limitations of solely examining peripheral blood immune subset levels, when it is likely that the pathogenic cells form only a minor population within any one subtype. It is therefore perhaps not surprising that peripheral blood CD4⁺, CD8⁺ and CD19⁺ cell

levels have not demonstrated biomarker activity to predict disease activation following immune reconstitution after CLAD and ALEM treatment²⁵, suggesting that further functional analysis may be required to define the mechanisms of action. Furthermore, without access to all of the primary data, we were unable to directly compare all of the subset analysis performed to clinical efficacy and safety, so their true relationship remains to be established. The lack of peripheral blood biomarker activity may also be due to compartmentalisation of autoimmunity outside of the peripheral blood. This could be due to the actions of immune cells sequestered within the CNS during MS, which trigger disease activity²⁴. Alternatively, compartmentalisation of the immune response within lymphoid tissues may also be a reason for the inability to detect a biomarker of autoimmune activity within the blood. As such it is of interest that sequestration of immune cells in lymphoid tissue, such as in the spleen and notably bone marrow, following spingosine-1-phosphate receptor modulation³⁵ may limit the capacity of ALEM to control MS³⁶, as ALEM may deplete less-effectively in lymphoid tissues compared to the blood^{36,37}. Alternatively, it is possible that the important pathogenic cells may not yet have been adequately analysed as it is likely they form only a small component within the pool of immune cells. Furthermore, it appears that in addition of any T cell inhibitory activity²³, most agents that inhibit MS also deplete memory B cells²⁴. Relating levels of memory B cells to therapeutic activity has yet to be performed in MS, but this has been used to personalise retreatment to limit relapse in other autoimmune diseases that are sensitive to CD20-depleting antibodies^{38,39}. Whilst it is clear that ALEM markedly depletes memory B cells²⁰, the memory B cell depleting capacity of CLAD is unknown. It is tempting to speculate that suppression MS disease-activity by CLAD may relate to long-term depletion of memory B cells. Further work, including functional studies and cytokine analysis, additional to that already known^{9,17,18} will be required to fully understand the definitive mechanism of action of these treatments, as both qualitative and quantitative changes in lymphocyte subsets will be important in defining their therapeutic activity^{9,18}.

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FIGURE LEGENDS**Figure 1.** *Cladribine targets mainly lymphocytes.*

Mean number of red blood cells and leucocytes following treatment with either placebo (n=42-101. Typically the lower limit of sample size was n=63, except week on 55) or a total doses of either 3.5mg/kg (n=47-103. Typically the lower limit of sample size was n=67, except on week 55) or 5.25mg/kg (n=38-104. Typically the lower limit of sample size was n=62, except on week 55) Placebo (Circle) or CLAD that was administered in monthly courses (Inverse triangle) at 0, 5 and 48 and 52 weeks (Diamond. 3.5mg/dose) and additionally at 9 and 13 weeks (Hexagon. 5.25mg/dose). The results show the mean \pm SEM of (A) Red blood cells (B) platelets (C) White blood cells, (D) Lymphocytes, (E) Monocytes, (F) polymorphonuclear neutrophils, (G) eosinophils or (H) basophils.

Figure 2 *Cladribine preferentially depletes B lymphocytes compared to a modest depletion of T cells.*

The results represent the mean percentage \pm SEM of blood lymphocytes compared to baseline following treatment with either placebo (Circle; n= 56-79) or total doses of either 3.5mg/kg (Diamond; n= 62-82) or 5.25/kg (Hexagon; n= 66-81) CLAD administered in monthly courses (inverse triangle) at 0, 5, 48 and 52 weeks (3.5mg/dose) and additionally at 9 and 13 weeks (5.25mg/dose). Results show the numbers of (A) CD3⁺ T cells, (B) CD19⁺ B cells, (C) CD4⁺ T cells (D) CD8⁺ T cells and (E) CD4⁺ naïve and (F) CD4⁺ memory T cells.

Figure 3 *The incidence of relapse does not related to peripheral blood CD3, CD4, CD8, CD19, T and B cell levels.*

CLAD was administered as weekly courses at 0, 5 and 48 and 52 weeks. The results represent the mean \pm SEM absolute number (per mm³) of peripheral blood: (A) CD3, (B) CD19, (C) CD4 (D) CD8 lymphocytes following treatment (inverse triangles) with oral CLAD in the CLARITY trial administered with 3.5mg/kg CLAD and those divided into groups that remained healthy (Circle. n=121-136/group) or those that had a least one relapse (Diamond. n=29-34).

Figure 4 *Depletion of lymphocyte subsets following alemtuzumab and cladribine treatment*

CLAD was administered as weekly courses of CLAD at 0, 5 and 48 and 52 weeks (Time of initiation of cycle. Inverse triangle) or weekly courses of alemtuzumab at 0 and 52 weeks. The results represent the mean absolute number of peripheral blood lymphocytes (per mm³) during the CLARITY trial in pwRMS treated with either placebo (circle; n= 68-80), CLAD 3.5mg/kg (grey diamond; n= 77-86), CLAD 5.25mg/kg (Hexagon; n= 79-84) and pwMS in the CARE-MS I trial treated with 12mg/day ALEM (Squares; n= 171-184).

FIGURE 1

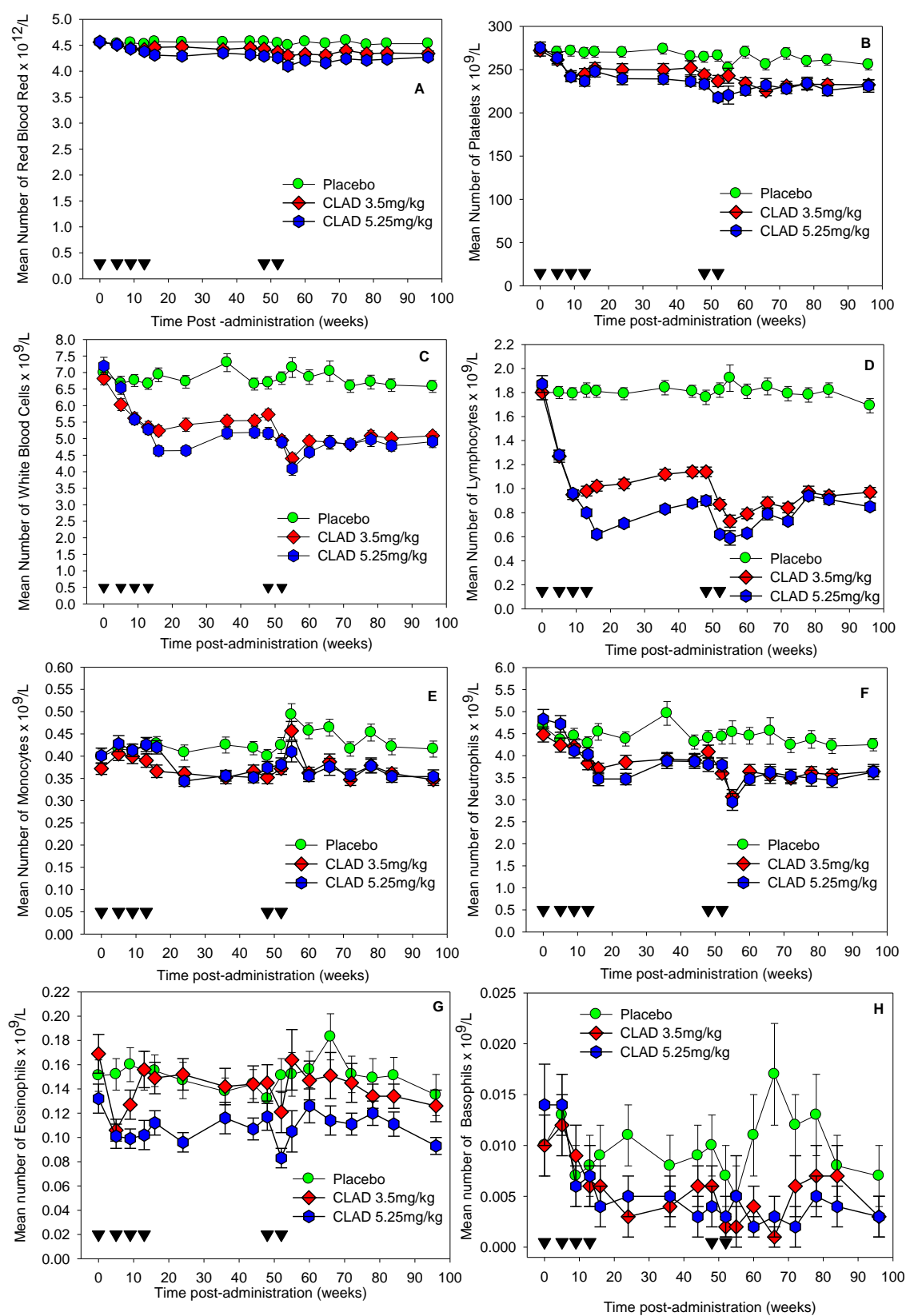


FIGURE 2

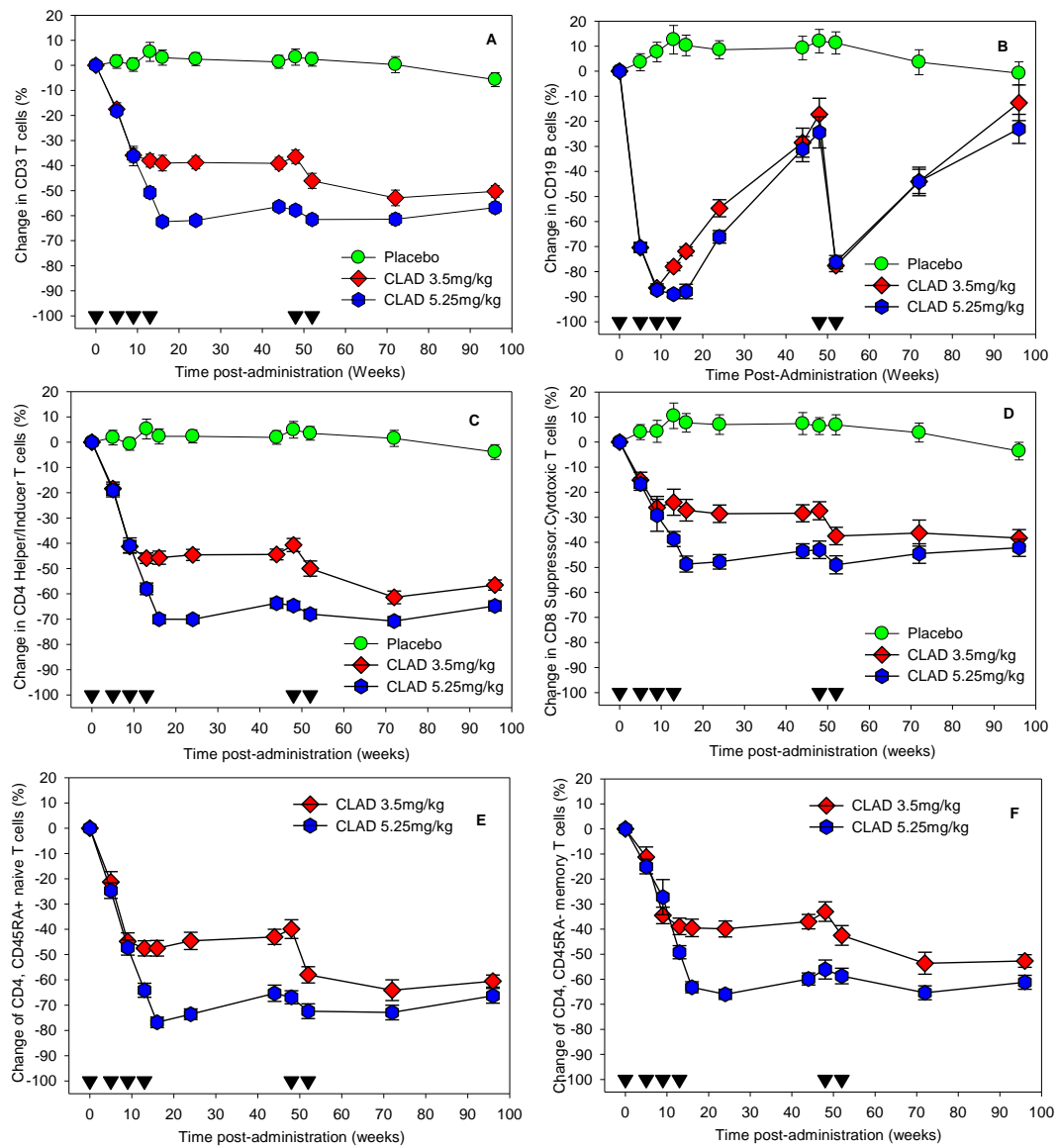


FIGURE 3

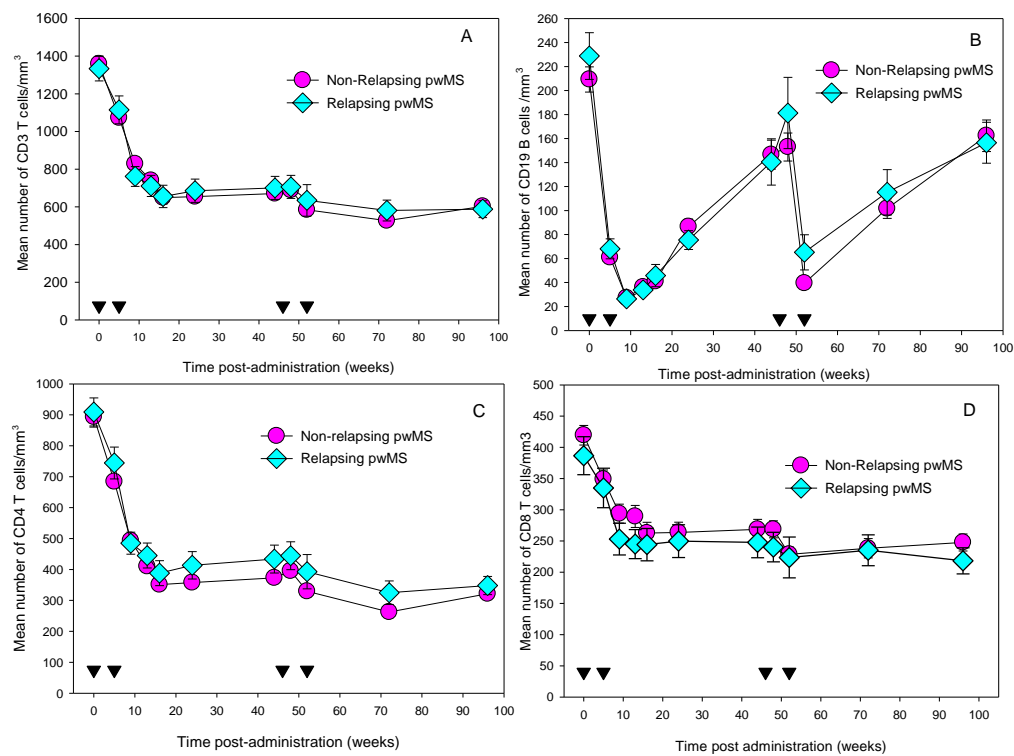
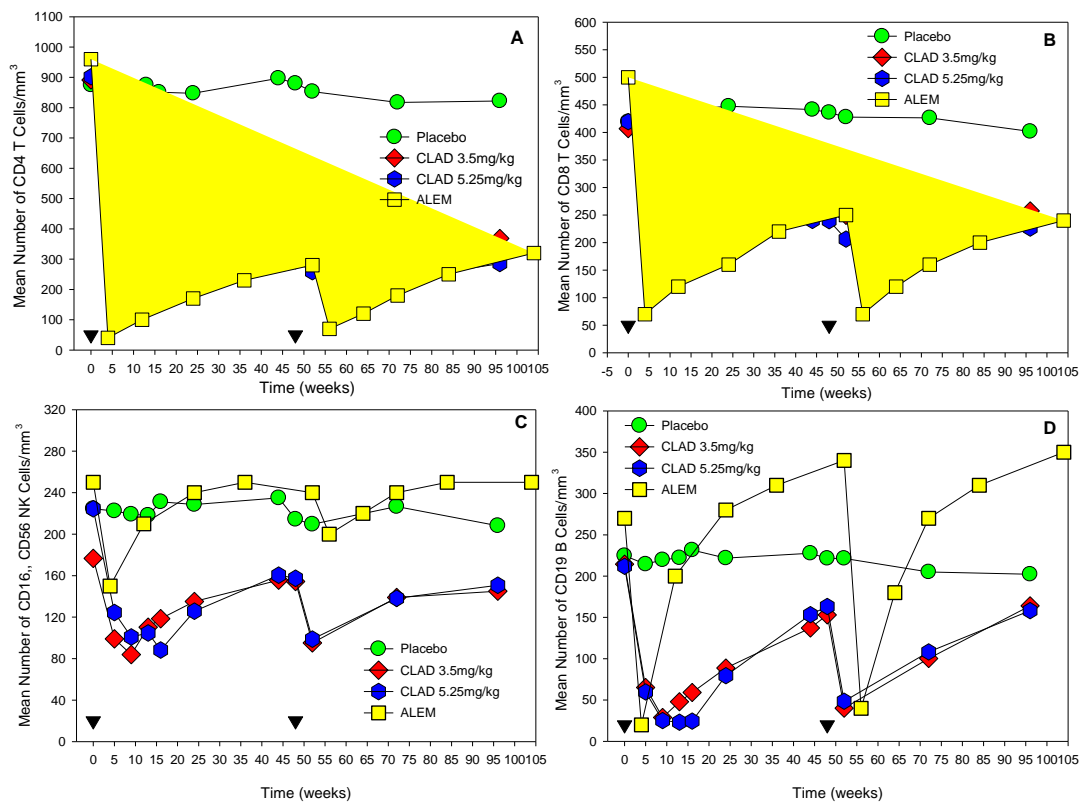


FIGURE 4



SUPPLEMENTARY DATA

Table e-1

Demographic and clinical characteristics of people participating in the CLARITY oral CLAD and the CARE-MS I alemtuzumab phase III trials, whose lymphocyte subsets were collected.

Trial Name	CLARITY			CARE-MS I
Variable	Placebo	Cladribine 3.5 mg/kg	Cladribine 5.25 mg/kg	Alemtuzumab 12mg/day
N pwMS	101	103	105	376
Age (yr)	39 ± 10	38 ± 11	41 ± 10	33 ± 8
Male	37 (37%)	32 (31%)	30 (29%)	133 (35%)
Female (%)	64 (63%)	71 (69%)	75 (71%)	243 (65%)
Weight (kg)	70 ± 16	69 ± 14	70 ± 16	73 ± 17
N white (%)	101 (100%)	102 (99.0%)	104 (99.0%)	352 (94%)
N black (%)	0 (0%)	0 (0%)	0 (0%)	11 (3%)
N other (%)	0 (0%)	0 (0%)	1 (1%)	12 (3%)
N pwMS previous DMT use (%)	31 (31%)	20 (19%)	29 (28%)	0 (0%)
Disease duration (yr)	9 ± 8	7 ± 8	10 ± 8	2 ± 1
Mean EDSS ± SD	3.2 ± 1.3	3.0 ± 1.3	3.3 ± 1.4	2.0 ± 0.8
N pwMS Gd+ lesions (%)	26 (26)	31 (30)	26 (25)	-
N Gd+ lesions on T ₁ MRI	0.7 ± 1.6	0.7 ± 2.1	1.0 ± 3.0	2.3 ± 5.1
T ₁ (Hypointense) Lesion volume	-	-	-	1.2±2.1 cm ³
T ₂ (Hyperintense) Lesion volume	14.2 ± 12.6 ml	15.6 ± 16.7 ml	17.1 ± 18.0 ml	7.4 ± 9.2cm ³

Demographic data from people having lymphocyte subset analysis from the phase III CLARITY trial of oral CLAD and the CARE-MS I trial of ALEM. The results report mean ± SD unless otherwise indicated. Differences between three arms of the CLARITY study are not significantly different as assessed by ANOVA/chi-squared analysis. Raw data from the CARE-MS-I trial were not supplied.

Table e-2

Safety and efficacy indices of pWRMS participating in CLARITY, and in whom lymphocyte subsets were collected.

Variable	Placebo (n=101)	CLAD 3.5mg/kg (n=103)	CLAD 5.25mg/kg (n=105)
Annualised relapse rate	0.40	0.18	0.16
Relative reduction vs. placebo*		55.4% p=0.01	61.0% p=0.004
ANOVA (Post hoc Tukey)	P=0.002 2v3 p= 0.953		
pWRMS with no relapse (%)	66 (65.3)	82 (79.6)	84 (80.0)
Odds ratio for CLAD vs. placebo**		2.07 p=0.022	2.12 p= 0.018
Chi Square p=0.022 Post-hoc Chi square	2v3 p= 0.944		
N patients with no relapse at 96 week N(%)	66 (65.3)	82 (79.6)	84 (80.0)
N patients with 1 relapse at 96 week N(%)	14 (13.9)	15 (14.6)	16 (15.2)
N patients with 2 relapses at 96 week N(%)	15 (14.9)	5 (4.9)	4 (3.8)
N patients with 3 relapses at 96 week N(%)	4 (4.0)	1 (1.0)	0 (0.0)
N patients with ≥4 relapses at 96 week N(%)	2 (2.0)	0 (0.0)	1 (1.0)
Mean no. of relapses at 96 weeks ±SD	0.65 ±1.1	0.27 ±0.60	0.27 ±0.62
ANOVA p=0.00048 (Post hoc Tukey)	1v2 p= 0.0021	1v3 p= 0.0017	2v3 p= 1.00
Rescue therapy. No. (%)	7 (6.9)	4 (3.9)	4 (3.8)
Odds ratio for CLAD vs. placebo* $\chi^2=0.50$		0.54	0.53
EDSS progression. N (%) $\chi^2=0.75$	19 (18.8)	16 (15.5)	16 (15.2)
pWRMS with new Gadolinium+ Lesions no. (%)	59 (58.4)	28 (27.2)	27 (25.7)
Post-hoc Chi square (p= 0.0021)	1v2 p= 0.0060	1v3 p= 0.0016	2v3 p=0.68
Mean no. new Gadolinium+ Lesions ± SD	0.41	0.15	0.15
ANOVA p=0.00035 (Post-hoc Tukey)	1v2 p= 0.0010	1v3 p= 0.0022	2v3 p= 0.97
Mean change in T2 weighted lesion volume	-12.1 ± 79.5	-15.1 ± 44.9	-33.3 ± 55.7
ANOVA p=0.035 (Post-hoc Tukey)	1v2 p= 0.94	1v3 p= 0.046	2v3 p=0.098
Adverse Events (N)	71 P=0.027	81	90
Moderate Adverse Events (N) vs. CLAD	39 p=0.018	47	54
Severe Adverse Events(N) vs. CLAD	7 p=0.29	6	12
Serious Infections vs. CLAD	18 p=0.72	23	22
Lymphopenia Reported as Adverse Event (N)	3 P<0.001	27	43

Post-hoc Tukey groups: 1= placebo, 2= CLAD 3.5mg/kg, and 3= CLAD 5.25mg/kg. * Odds of event occurring were calculated as the number of patients with the event divided by those without the event. The odds ratio was calculated as the odds in the groups treated with CLAD divided by the odds in the placebo group. **The relative reduction was calculated as the ratio of the difference in the measures in the groups treated with CLAD and placebo group to the measure of the placebo group.